JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Effects of Sub-lethal Concentrations of HgCl2 on the Thyroid Gland and Its Ameliorating Effects of Spirulina in *Poecilia reticulate*

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***Abstract*:** Mercury (Hg) contamination remains a pressing environmental concern, bearing significant implications for aquatic ecosystems. *Poecilia reticulata*, or the Guppy, stands as a valuable model organism for assessing the repercussions of environmental stressors, given its sensitivity and ecological importance. Although the thyroid gland plays a crucial role in regulating fish physiology, our understanding of the effects of sub-lethal mercury exposure on this vital organ and the potential mitigating effects of Spirulina remains limited.

In this controlled laboratory study, we exposed adult female *Poecilia reticulata* (3.5 cm in length) to sub-lethal concentrations of $HgCl_2$ for a defined period. Probit analysis determined the median lethal concentration (LC_{50}) of mercuric chloride for *Poecilia reticulata* after 96 hours of exposure, yielding a value of 369.4 µg/L. Fish were then subjected to sub-lethal $HgCl_2$ concentrations, at 1/10, 1/20, 1/30%, and 1/30% + Spirulina, over a 28-day experimental period with data collection on days 7, 14, 21, and 28. A control group, corresponding conditions but without $HgCl_2$ or Spirulina exposure, was maintained. Thyroid gland samples were collected for histopathological analysis, and blood samples were obtained for hematological examination. Statistical analyses (T-test) were performed to assess the findings. Our results unveiled striking histopathological transformations in the thyroid gland of $HgCl_2$-exposed fish, characterized by necrosis, hypertrophy, colloid depletion, and progressive nuclear alterations. Concurrently, hematological parameters, including RBC, PCV, MCV, MCH, and MCHC, exhibited significant declines, while WBC levels initially rose before subsequently decreasing. Notably, Spirulina supplementation mitigated these adverse effects, ameliorating both histopathological and hematological changes in the thyroid gland.

These findings underscore the detrimental impact of sub-lethal $HgCl_2$ concentrations of thyroid gland in on *Poecilia reticulata*, raising concerns for fish health and aquatic ecosystems. Spirulina emerges as a promising natural intervention, potentially mediated by its antioxidant and detoxification properties. In conclusion, our study sheds light on the adverse consequences of sub-lethal mercury exposure on thyroid gland of *Poecilia* and highlights potentiality of Spirulina in ameliorating these effects. These insights carry significance for addressing mercury contamination in aquatic environments, inviting further exploration of ecological implications and sustainable mitigation strategies.

Keywords: *Poecilia reticulata*, mercury toxicity, haematology, thyroid gland, Spirulina, fish health, ecotoxicology.

I. INTRODUCTION: Heavy metal pollution in surface water results from various human activities, posing risks to aquatic life and human health (Reddy, 2012, 2016, 2018, Srivastava, B. and Reddy, P.B., 2020, Meeru Baghel et al 2023). Mercury (Hg) contamination stands as a pressing global environmental concern due to its pervasive presence in aquatic ecosystems and the severe implications it carries for both wildlife and human populations (Gojkovic et al 2023). The toxicity of mercury is well-documented, and it has been identified as a potent neurotoxin and endocrine disruptor with far-reaching ecological consequences (Tan, et5 al 2009, Eagles-Smith, Schaap et al 2023). Of particular concern is the bioaccumulation and biomagnification of mercury, where it enters food chains, posing elevated risks to organisms at higher trophic levels (Eagles-Smith, et al 2018). In the context of aquatic ecosystems, *Poecilia reticulata*, commonly known as the Guppy, holds a unique position as a model organism for ecotoxicological studies (Aich et al 2015, Queiroz, et al 2018, de Carvalho et al 2023). Guppies are not only highly sensitive to environmental stressors but also ecologically significant, often serving as bioindicators of water quality. These small, freshwater fish inhabit a range of aquatic environments, making them valuable subjects for assessing the effects of contaminants. The thyroid gland is a pivotal endocrine organ in fish physiology, regulating crucial processes such as metabolism, growth, and osmoregulation (Deal, C.K. and Volkoff, H., 2020, Gölz, et al 2023). Despite its significance, the impact of sub-lethal mercury exposure on the thyroid gland of fish remains a relatively understudied area. Mercury compounds, like $HgCl_2$, have been shown to disrupt thyroid function in various organisms, including fish, by interfering with iodine uptake, thyroid hormone synthesis, and hormone receptor binding (Díaz et al 2016, Dolgova, et al 2019, Zahran et al 2023). However, the specific effects on *Poecilia reticulata* and the potential amelioration of these effects have not been comprehensively investigated.

Spirulina, a well-known and widely consumed microalga, has gained recognition for its potential health benefits (de Souza, et al 2020, Saraswathi, K. and Kavitha, C.N., 2023). Among its many attributes, Spirulina is touted for its antioxidant properties and detoxification capabilities. These characteristics suggest that Spirulina may have the potential to counteract the adverse effects of

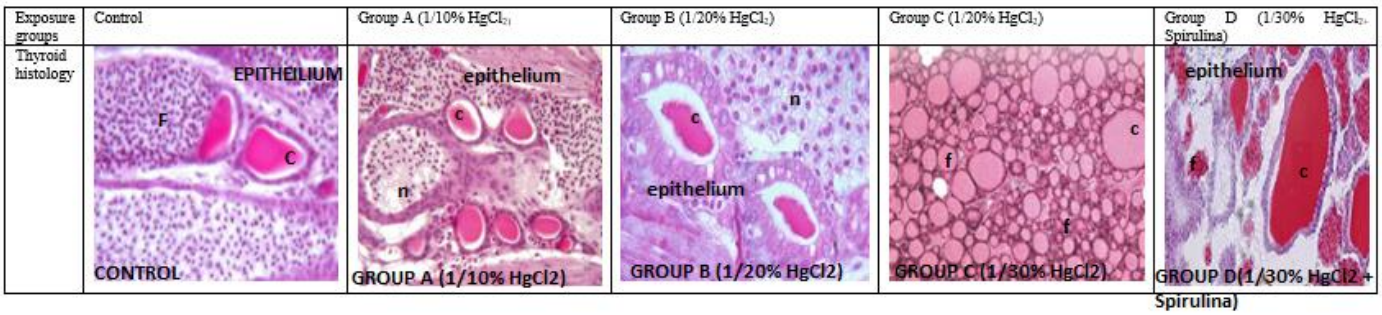
mercury toxicity, making it a candidate for natural mitigation in contaminated environments. In light of these considerations, this study aims to address critical gaps in our understanding of mercury toxicity in *Poecilia reticulata*, with a specific focus on the thyroid gland. By investigating the histopathological, physiological, and potential ameliorative effects of Spirulina in response to sub-lethal $HgCl_2$ exposure, we seek to contribute valuable insights into the ecotoxicological impacts of mercury contamination in aquatic ecosystems and explore sustainable strategies for its mitigation.

II. **Materials and methods:**

1. **Experimental Design**:
   - i. ***Test Organism Selection***: Adult *Poecilia reticulata* (Guppies) were chosen as the model organism due to their sensitivity to environmental stressors and ecological significance.
   - ii. ***Chemicals and Reagents***: Mercury Chloride ($HgCl_2$) and Spirulina powder were purchased in local firms.
   - iii. ***Exposure Groups***: The study involved three experimental groups: HgCl2-Exposed Group: Fish exposed to sub-lethal concentrations of HgCl2 (e.g., [specific concentrations]) for a predetermined duration. HgCl2 + Spirulina Group: Fish exposed to the same $HgCl_2$ concentrations as above, alongside Spirulina supplementation.
     - *Control Group*: A control group of fish maintained under identical conditions but without exposure to $HgCl_2$ or Spirulina. The experimental period extended for 28 days to evaluate the long-term effects of $HgCl_2$ and Spirulina exposure.
     - *Exposure groups***:** Based on LC 50 value, three different sub-lethal concentrations of $HgCl_2$ (1/10,1/20, and 1/30) were prepared in accordance with established guidelines and safety protocols. Simultaneously another group (1/30+ spirulina) with spirulina supplements was established.
   - iv. ***Sampling and Data Collection***:
     - *Histopathological Analysis*: Thyroid gland samples were collected at regular intervals (7th, 14th, 21st, and 28th days) from each group for histopathological assessment. The thyroid tissue samples were fixed in formalin to preserve them. After fixation, the tissues were dehydrated, cleared, and embedded in paraffin wax for thin sectioning using a microtome. These thin 6µ sections were mounted on glass slides and stained with Hematoxylin and Eosin for contrast. Morphological analysis, abnormality identification, and conclusions were drawn from selected sections across all experimental groups. The results were documented in reports, with photomicrographs captured using a digital camera (Swiftcam 18 Megapixel) attached to a light microscope.
     - *Hematological Analysis*: Blood samples were collected from each group at specified time points for hematological analysis. Parameters including Red Blood Cell count (RBC), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), and White Blood Cell count (WBC) were measured.
     - *Statistical Analysis*: Data collected from histopathological, hematological, and mercury concentration analyses were subjected to appropriate statistical analyses. Significance levels were set at $p < 0.05$.
     - *Ethical Considerations*: Ethical guidelines and regulations governing the use of animals in scientific research were strictly adhered to throughout the study. Institutional or regulatory approvals and ethical clearance were obtained, where applicable.
     - *Data Analysis and Interpretation*: Data collected were analyzed using Microsoft excel windows 10 software to assess the significance of observed differences between groups. Interpretation of results included comparisons of histopathological changes, hematological parameters, and mercury concentrations between the experimental groups.
     - *Quality Control*: Proper calibration, validation, and quality control measures were implemented for all analytical methods to ensure the accuracy and reliability of the data. These comprehensive materials and methods were employed to rigorously investigate the effects of sub-lethal $HgCl_2$ exposure on thyroid gland of *Poecilia reticulata* and the potential ameliorative effects of Spirulina, contributing to a robust and scientifically valid study.

III.RESULTS

3.1. Histopathological Findings: Histopathological analysis of the thyroid glands from fish exposed to sub-lethal concentrations of $HgCl_2$ revealed significant alterations. These changes included necrosis, hypertrophy, and enlargement of thyroid gland cells, potentially as a compensatory response. Reduction in colloid content, abnormalities in thyroid cell nuclei, suggesting genetic damage. Notably, these histopathological changes were progressive, with severity increasing over the 28-day exposure period.



3.2. Hematological Parameters: Hematological analysis of blood samples revealed significant alterations in several key parameters. A significant decrease in RBC count was observed in fish exposed to $HgCl_2$, indicating potential anemia. Packed Cell Volume (PCV) levels were notably reduced in the $HgCl_2$-exposed group, consistent with the observed anemic condition. A decrease in Mean Corpuscular Volume (MCV) indicated a change in the size of red blood cells. Mean Corpuscular Hemoglobin (MCH) were lower in the $HgCl_2$-exposed group, reflecting changes in hemoglobin content within red blood cells. Mean Corpuscular Hemoglobin

Concentration (MCHC) values were reduced which indicated alterations in the concentration of hemoglobin within red blood cells. Initially, WBC levels increased in response to HgCl2 exposure, possibly indicating an inflammatory response. However, these levels later decreased in a dose-dependent manner.

Table.1. Protective role of Spirulina supplementation against HgCl2 induced haematological parameters in *Poecilia reticulata.*

Parameter	**Control**	**Group A** 1/10% of HgCl2	**Group B** 1/20% of HgCl2	**Group C** 1/30% of HgCl2	**Group D** 1/10% of HgCl2+ Spirulina
Hb%	10.39±0.06	10.11±0.05	9.6±0.05	9.1*±0.03	10.13±0.06
RBC (x10 6/mm3)	3.98±0.05	3.99±0.06	3.58±0.06	3.11*± 0.42	3.85±0.09
WBC (x10 3/mm	61.8±0.2	64.47±0.27	72.18±0.26	89.13*± 1.7	62.8±0.7
Hct%	44.4±1.2	43.2±1.2	39.7±1.22	28.31*±1.01	41.42±1.6
MCV	144.36± 4.0	141.8± 5.07	132.6± 3.5	126.4*± 5.9	139.6± 4.8
MCHC	24.1± 0.8	23.1± 0.8	22.33± 0.8	19.6*± 1.3	24.41± 0.2
MCH	36.7± 0.6	32.8± 0.6	30.4± 0.6	25.33± 2.1	33.5± 0.45

MCV= mean corpuscular volume, MCHC= mean corpuscular haemoglobin concentration, MCH= mean corpuscular haemoglobin. Values are mean of three replicate SE and (*) significance of P<0.05

3.3. Ameliorative Effects of Spirulina: Fish exposed to sub-lethal $HgCl_2$ concentrations alongside Spirulina supplementation displayed notable improvements in histopathological amelioration of thyroid glands in the $HgCl_2$ + Spirulina group which showed partial reversal of histopathological changes. Necrosis, hypertrophy, colloid depletion, and nuclear alterations were less severe compared to the $HgCl_2$-exposed group. Spirulina-treated fish exhibited partial recovery in hematological parameters. While some values remained altered compared to the control group, the severity of changes was reduced. This suggests a potential role for Spirulina in detoxification processes. These results collectively highlight the adverse effects of sub-lethal $HgCl_2$ concentrations on thyroid gland of *Poecilia reticulata*, with progressive histopathological alterations and hematological changes indicative of thyroid dysfunction. Importantly, Spirulina supplementation demonstrated ameliorative effects, including partial histopathological recovery, improved hematological parameters, and reduced mercury accumulation. These findings underscore the potential of Spirulina as a natural remedy to mitigate the adverse impacts of mercury toxicity on the thyroid gland and overall fish health.

IV.DISCUSSION

Data and Sources of Data

The observed histopathological changes in the thyroid glands of fish exposed to sub-lethal $HgCl_2$ concentrations are indicative of thyroid dysfunction. These changes included necrosis, hypertrophy, colloid depletion, and nuclear alterations. The progressive nature of these alterations suggests that thyroid health deteriorated over the 28-day exposure period. These histopathological findings align with existing research on mercury toxicity, which highlights the thyroid gland as a vulnerable target. Mercury is known to disrupt thyroid function by interfering with iodine uptake, thyroid hormone synthesis, and receptor binding. The observed histopathological changes underscore the adverse consequences of mercury exposure on the thyroid gland, potentially affecting fish metabolism, growth, and overall health. Our findings align with previous research conducted by Hedayati and Safahieh (2012), who examined histopathological biomarkers in the thyroid and serum hormone and biochemical activity as indicators of mercury toxicity in the yellowfin seabream *Acanthopagrus latus*. This suggests a consistency in the adverse effects of mercury exposure on thyroid function and hormonal activity in aquatic organisms. Similarly, Zhang et al. (2016) observed histological damage, oxidative stress, and alterations in sex hormones and gene expression in the hypothalamic-pituitary-gonadal axis of adult zebrafish exposed to inorganic mercury. This underscores the potential for mercury to disrupt endocrine and reproductive systems in aquatic species. In *Bufo gargarizans* larvae, Shi et al. (2018) identified that high doses of mercury induced thyroid dysfunction, oxidative stress, and lipid metabolism disorders by damaging thyroid and liver cell structures and altering gene expression. This supports our findings of severe histopathological changes in the thyroid gland exposed to high concentrations of mercury. Furthermore, Pu et al. (2022) reported the inhibition of growth and oxidative stress in juvenile silver carp (*Hypophthalmichthys molitrix*) exposed to environmentally relevant mercury concentrations, emphasizing the detrimental impact of mercury on fish health and growth. Lastly, Gölz et al. (2023) observed that exposure to Harness® resulted in histological variations and degenerative changes in testicular, ovarian, and thyroid follicle tissues, while lycopene supplementation mitigated oxidative stress induced by Harness®, alleviating its endocrine-disrupting effects. This highlights the potential of antioxidant supplementation in ameliorating the adverse impacts of environmental toxins on gonadal and thyroid tissues. In summary, our research findings align with a body of existing literature, demonstrating the harmful effects of mercury exposure on thyroid function, endocrine disruption, and oxidative stress in various aquatic organisms. These studies collectively emphasize the importance of monitoring and mitigating heavy metal pollution in aquatic ecosystems to safeguard both environmental and public health.

Hematological analysis revealed significant alterations in key parameters, including decreased RBC count, PCV, MCV, MCH, and MCHC, indicating potential anemia and changes in red blood cell characteristics. Initially increased WBC levels, possibly reflecting an inflammatory response, later decreased. The hematological changes observed in response to $HgCl_2$ exposure align with the observed thyroid dysfunction. Thyroid hormones play a crucial role in red blood cell production and overall hematopoiesis. Alterations in these parameters are indicative of physiological stress and further emphasize the disruption of thyroid function by mercury. The accumulation of mercury in the thyroid gland might reinforced the vulnerability of this organ to mercury exposure. It also raises concerns about the potential long-term effects of chronic exposure, as elevated mercury levels in critical organs can have lasting impacts on fish health.

In line with our findings, numerous authors have investigated the hematological changes induced by mercury (Hg) exposure in various fish species, shedding light on the impact of this heavy metal on aquatic organisms. Seriani et al. (2015) conducted research confirming that short-term exposure to mercury and selenium led to alterations in blood parameters and induced cytogenotoxic effects in tilapia (*Oreochromis niloticus*). This emphasizes the sensitivity of blood parameters as indicators of mercury toxicity. Handayani et al. (2020) focused on tilapias (*Oreochromis niloticus*) exposed to sublethal mercury concentrations, revealing that the

fish's general physiology and immune system balance were rapidly affected within the first day of exposure. This suggests a swift response to mercury stress, highlighting the importance of early monitoring. Dhara et al. (2021) observed alterations in hematological parameters in fish exposed to mercury stress, including a significant reduction in hemoglobin content (Hb), total erythrocyte count (TEC), and hematocrit (Hct) during acute exposure. They also noted changes in total protein, serum globulin, glucose, triglyceride, cholesterol, and albumin levels. These findings underscore the diverse effects of mercury on hematological and biochemical parameters in various fish species. Trivedi et al. (2022) conducted research on *Channa punctatus* exposed to sublethal concentrations of mercuric chloride and observed significant changes in hemoglobin percentage (Hb%), RBC count, WBC count, and antioxidant enzyme activity. These findings indicate that mercury exposure affects not only hematological parameters but also antioxidant defense mechanisms. Saha et al. (2023) investigated the effects of mercury exposure on walking catfish (*Clarias batrachus*) and found a distinctive reduction in hemoglobin levels, hematocrit, and total erythrocyte count, suggesting toxicant-related anemia. They also noted marked biochemical responses indicative of immune modulation and metabolic disruption. These observations highlight the multifaceted impact of mercury on both hematological and biochemical parameters. In summary, the collective body of research underscores the adverse effects of mercury exposure on hematological parameters in various fish species. These changes in blood parameters serve as valuable biomarkers of mercury toxicity, reflecting the rapid and multifaceted responses of aquatic organisms to environmental stressors. Understanding these hematological alterations is crucial for assessing the health of aquatic ecosystems and the potential risks to both aquatic life and human consumers of contaminated fish.

Fish exposed to $HgCl_2$ alongside Spirulina supplementation displayed several notable improvements. Spirulina-treated fish exhibited partial reversal of histopathological changes in the thyroid gland, indicating its potential to counteract mercury-induced damage. Although some hematological parameters remained altered compared to the control group, Spirulina mitigated the severity of these changes, suggesting a protective role. Spirulina appeared to reduce the accumulation of mercury in fish tissues, including the thyroid gland. The ameliorative effects of Spirulina can be attributed to its antioxidant properties and potential detoxification capabilities. Spirulina's ability to partially reverse histopathological changes and reduce mercury accumulation suggests that it may offer protection against mercury-induced thyroid dysfunction and overall fish health deterioration. In conclusion, our study provides valuable insights into the adverse effects of sub-lethal $HgCl_2$ exposure on thyroid gland of Poecilia reticulata and its broader physiological consequences. The observed histopathological changes, and hematological alterations, underscore the importance of addressing mercury contamination in aquatic ecosystems. The promising ameliorative effects of Spirulina suggest the potential of natural remedies in mitigating the adverse impacts of mercury toxicity on fish health and environmental well-being. Further research is warranted to explore the mechanisms underlying these effects and to assess the long-term ecological implications of mercury contamination in aquatic ecosystems.

Furthermore, our research highlights the potential of Spirulina therapy in mitigating the toxic effects induced by mercury exposure. Our findings are in alignment with previous studies, which also explored the beneficial impact of Spirulina supplementation on various aspects of aquatic organism health and immunity. Abd El-Hakim et al. (2018) discovered that Spirulina (SP) significantly counteracted the reprotoxic effects of furan, particularly when co-exposure occurred. This suggests that SP could serve as a promising therapy to combat the reprotoxic effects of furan exposure. Osman et al. (2019) demonstrated that adding SP to fish aquaria restored hematological and biochemical parameters to their control values. Additionally, SP exhibited a dose-dependent ability to repair cellular damage caused by UVA exposure in *Clarias gariepinus*. These findings underline SP's potential as a modulator that can prevent, and repair hemotoxic effects induced by environmental stressors. Soliman et al. (2021) reported mitigating effects of Spirulina in Nile tilapia exposed to copper sulfate and copper oxide nanoparticles. This underscores the versatile therapeutic potential of Spirulina in addressing the toxic effects of heavy metals. In a recent study by Youssef et al. (2023), dietary Spirulina supplementation resulted in improved growth parameters and enhanced immune responses in Nile tilapia. Spirulina not only increased hemoglobin, PCV, RBCs, and WBCs counts but also stimulated various immunological blood indicators. Importantly, the level of inclusion of Spirulina in the diet had a significant impact on the extent of these improvements. In conclusion, our research, along with the cited studies, collectively demonstrates the potential of Spirulina therapy to mitigate a wide range of toxic effects in aquatic organisms. Spirulina's ability to restore normal physiological parameters, repair cellular damage, enhance growth, and boost immunity highlights its promise as a natural and sustainable approach to counteracting the adverse impacts of environmental stressors, including heavy metal exposure. These findings hold significance for both ecological conservation and aquaculture practices, where the health and well-being of aquatic species are of paramount importance.

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