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# Assessment of Harmful Food Colorants Induced Aquatic Toxicity and in General Health Issues of Aquatic Organisms and Finding the Solutions.

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**ABSTRACT**

**Background:** Environmental stress in both wild and aquaculture conditions is faced by Channa fish. Chemical stressor, such as Allura Red, a synthetic dye, was reported to have toxicological effects on aquatic organisms. This study explores the potential protective role of folic acid against histopathological damages caused by food color Allura Red stress in Channa fish.

**Methods:** Study employed a controlled experiment where Channa fish were subjected to different concentrations of Allura Red, then supplemented with folic acid. Tissue damage in gills, liver, and kidneys was determined by histopathological analyses.

**Results:** The results indicated that there were histopathological alterations in tissues exposed to food color Allura Red, including necrosis and inflammation in the gills and liver. However, folic acid treatment showed a marked reduction in these damages, restoring the integrity of the tissues. The analysis also included statistical comparison of tissue damage scores across different experimental groups..

**Conclusion**: The results suggest that folic acid possesses therapeutic potential in countering the toxic effects of Allura Red stress on Channa fish. Such results may have further implications in the management of aquaculture and aquatic ecosystem health management.

**Keywords**:, Allura Red, Aquaculture, *Channa* fish, Folic acid, Histopathology, Oxidative stress, Tissue damage

## INTRODUCTION

Recently, synthetic dyes such as Allura Red (AR) have been widely used in food and textile industries, but its use in aquatic environments is a concern because it could potentially harm aquatic animals. It has the potential to form reactive oxygen species (ROS), causing oxidative stress, which then damages the tissue and results in organ dysfunction among the aquatic animals. The most prevalent species of fish cultivated in Asian aquaculture, Channa fish, is known to be highly sensitive to environmental pollutants among which synthetic dyes. Histopathological damages done by AR in Channa fish have also been documented earlier showing alterations like cell necrosis, inflammation, and disruption of organ structure. However, the potential for supplementation with nutritional supplements has not been studied in detail to delay these effects.

A water-soluble B-vitamin, folic acid is highly involved in the metabolism of cellular components, synthesis, and DNA repair. In fact, the antioxidant properties reported by several researchers indicate its capacity to neutralize ROS and its protective role against oxidative stress. While folic acid's mammalian role has been studied intensively, such effects in fish, especially during environmental stress, have not received much attention so far. This study will try to fill the gap by evaluating the histopathological effect of AR on Channa fish and whether supplementation with folic acid can help reduce the damage induced.

Previous studies have shown that antioxidants such as vitamins C and E help to mitigate the impact of pollutants on aquatic organisms, but folic acid has not been investigated as a possible agent in mitigating the impact of dye stress on fish. This study will examine the potential protective role of folic acid supplementation in Channa fish subjected to AR-induced stress, hence opening up new avenues for the use of folic acid in aquaculture and environmental conservation.

Several studies [1][2][3] have already established the impact of synthetic dyes on aquatic health, while others [4][5] have explored the protective mechanisms of antioxidants in aquatic organisms. The current research builds on these foundations by investigating the specific effect of folic acid in mitigating the adverse outcomes of Allura Red stress on Channa fish. We hypothesize that folic acid supplementation can alleviate oxidative damage and histopathological changes, promoting tissue recovery and improving overall fish health.

## MATERIALS AND METHODS

- **Experimental Design**:

  The study was conducted using 25 *Channa* fish (average weight: 25g) obtained from a local fish farm. The fish were acclimatized for 7 days under standard laboratory conditions before the start of the experiment.

- **Group Division**:

  Fish were randomly assigned to four groups (n = 30 per group):

  - Group 1: Control (no exposure to AR)
  - Group 2: AR exposure (0.144 & 0.216 gm/L)
  - Group 3: AR exposure + Folic acid (Equivalent dose)
  - Group 4: Folic acid only ( same as AR)

- **Allura Red Exposure**:

  Food color used here is Allura red(AR). for the basic experiment to assess the harmful impact of food dye in aquatic environment.AR was dissolved in distilled water and administered to the designated groups for a period of 14 days. Lethal concentration (LC50) was 0.080 gm/L. The exposure concentration of 0.144 gm/L,0.216gm/L was selected based on previous studies that have shown significant histopathological changes at this level.

- **Folic Acid Supplementation**:

  Folic acid was supplemented with the equivalent dose strategy through the water so that there was constant exposure to the solution during the duration of the experiment. The concentration of folic acid used was determined from previous studies concerning fish nutrition.

- **Tissue Collection and Histopathological Analysis**:

  Fish were euthanized at the end of the exposure period. Tissues of gills, liver, and kidney were dissected out and fixed in 10% formalin. The tissue samples were processed, sectioned, and stained with Hematoxylin and Eosin (H&E) to be examined for histopathological changes under a light microscope.

- **Statistical Analysis**:

  Histopathological scores were done based on severity of damage and statistical analysis by ANOVA followed by Tukey's post-hoc test to compare differences between experimental groups.

## RESULTS

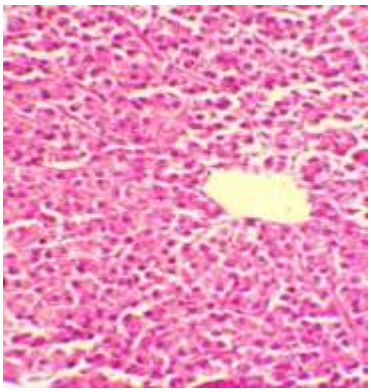
In Allura Red exposed fish, it was seen under histopathological analysis that considerable changes occurred in the gills, liver, and kidneys. The epithelial cells in the gills exhibited swelling and necrosis with evident signs of inflammation. In the liver, extreme vacuolization and necrosis of the hepatocytes, along with the infiltration of inflammatory cells, were seen. Kidney tissues indicated tubular necrosis and damage to the glomeruli.

Complementarily, the histopathological changes appeared to be significantly decreased in folic acid-supplemented fish. The integrity of the epithelium in the gill tissue was improved, with fewer necrotic and inflammatory changes. In the liver, vacuolization and necrosis were significantly reduced, and the kidney showed less tubular damage and glomerular preservation. These findings suggest a protective role for folic acid against the toxic effects of AR.

**Table 1: Liver damage scores in experimental groups**

| **Group** | **Score (Mean ± SD)** |
|---|---|
| Control | 1.1 ± 0.2 |
| AR exposure | 5.0 ± 1.5 |
| AR + Folic | 2.1 ± 0.7 |

| | |
|---|---|
| Folic only | 1.4 ± 0.3 |



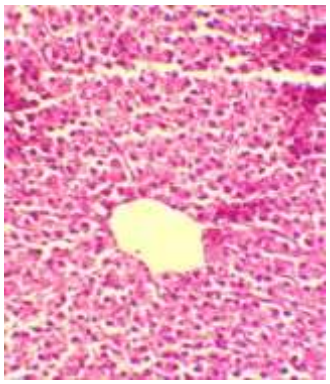
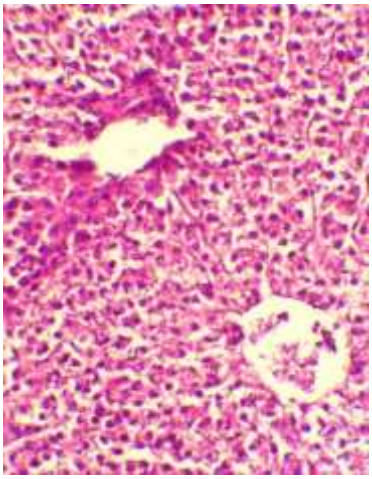


**Figure.1a Control fish** **Figure.1b Control Folic Acid [0.216 gm/L]**



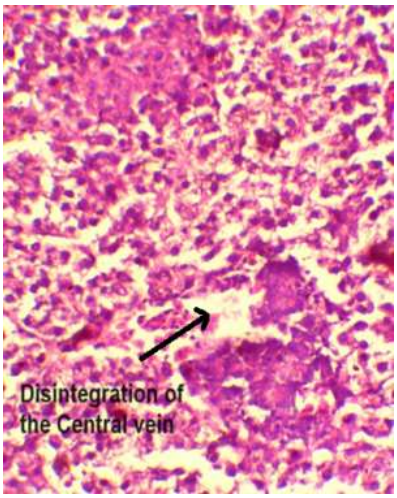
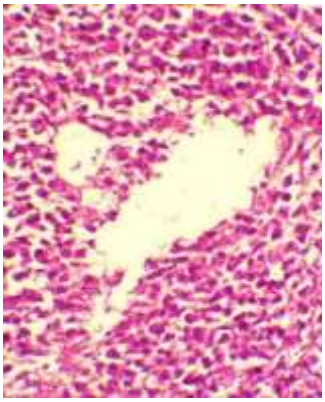



**Figure.1c 2X AR [0.144 gm/L]** **Figure.1d 3X AR [0.216 gm/L]**



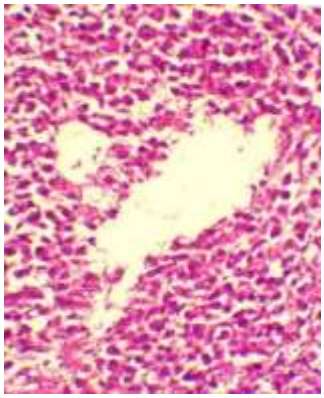


**Figure.1e 2X AR [0.144 gm/lt] + FA [0.144 gm/L]** **Figure.1f 3X AR [0.216 gm/lt]+FA [0.216 gm/L]**

**Figures1 a-f Reflects the various histological changes as observed in Liver tissue from the various experimental groups.**

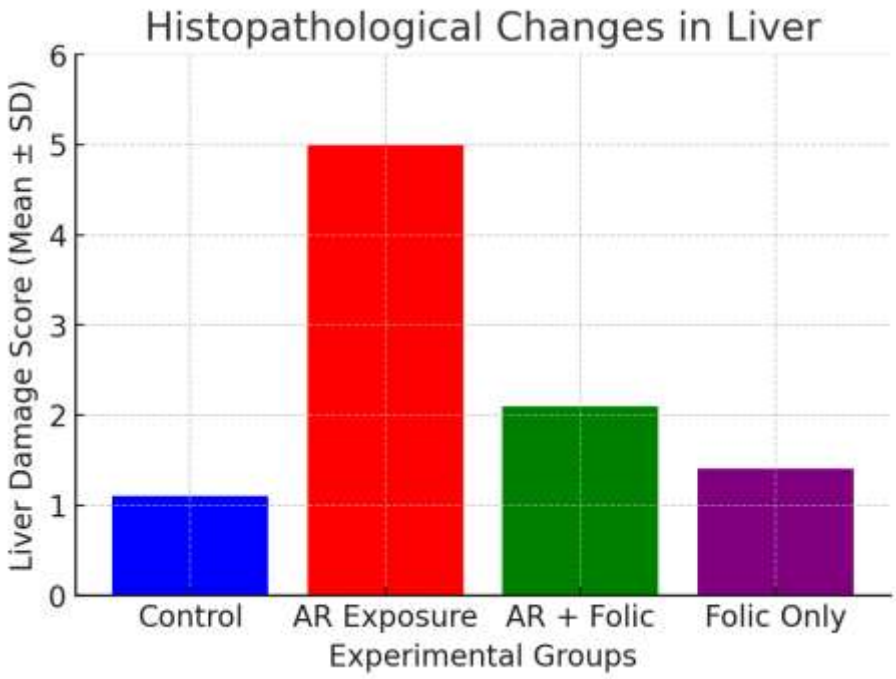



**Figure 2: Graphical Presentation of Liver damage score and AR exposure doses**

## DISCUSSION

Results herein showed the degree of histopathological damage presented by Allura Red exposure on Channa species. This experiment confirms previous evidence suggesting that the presence of artificial dyes exerts harmful results to aquatic-life. The highly positive changes evidenced in the improvement of liver tissues revealed after folic acidsupplementation points to the plausible use of the said vitamin in exerting such an antioxidant activity. This study adds its weight to growing literature on aquatic species showing support for the idea that folic acid may contribute to the mitigating of damage caused by oxidative stress.

Previous studies have demonstrated that folic acid has antioxidative properties that help neutralize ROS, and its role in enhancing DNA repair mechanisms may contribute to tissue recovery. In this study, folic acid supplementation seems to counteract the histopathological changes induced by AR exposure, possibly through the reduction of oxidative stress and promotion of cellular repair. This is consistent with findings in other species where folic acid has been shown to improve cellular health and tissue regeneration.

These results of this study have significant implications for aquaculture practices, especially in those environments where synthetic dyes and other pollutants are prevalent. Even though the present study was carried out on Channa fish, similar results could be obtained with other fish species exposed to environmental stressors, making folic acid a good candidate for enhancing the health of aquatic organisms under pollutant stress.

## CONCLUSION

In conclusion, we may say that supplementation with folic acid has a significant potential in alleviating the histopathological damages induced by Allura Red exposure in Channa fish. This study reveals the role of folic acid as a protective agent against oxidative stress, thereby improving the health and sustainability of aquaculture systems. Future research should explore the underlying mechanisms of folic acid's protective effects and its potential application across various aquatic species exposed to environmental pollutants.

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Additional addendum

**1. Ethical Approval**

This study was approved by the ethical research committee board of Studies, GMRD College, Samastipur, Bihar. Since , the entire experiment was done on edible fish available to market so no additional approval was required from Animal Rights Board of India.

**2. Acknowledgement**

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**3. Conflict of interest**

The authors declare that no conflict of interest among the contributing authors of this paper is there.

**4. Funding statement**

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors." (NB: this does not apply to protocols).

The research object chemical Allura red was procured from the local market, Kermy synthetic food sellers & all the required reagents were provided by the Department of Zoology, GMRD college Samastipur, Bihar.

**5. Authors' Contribution**

The entire research was conducted by the corresponding author , Supriyo Acharya with the supervision of Dr. Paramesh Chaudhari and Dr. M.S. Gaikwad.

**6. Data Availability**

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.