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Research article

Histopathological Effects of Allura Red (E129) Food Colorant on Liver and Kidney Tissues of Male Albino Mice

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Abstract

Background: Synthetic food colorants are widely found in food products to improve their visual appeal. However, the potential health risks associated with these colorants have raised serious health concerns. **Aim:** This study aimed to investigate the pathological effects of Allura Red food colorant on the liver and kidney of male albino mice in terms of changes in relative weights of these organs and its histopathological impact on their tissues. **Methods:** Thirty male albino mice were randomly divided into three groups of 10 animals each and received daily oral doses of Allura Red at doses of 700 and 1400 mg/kg body weight for 42 days. At the end of the study, liver and kidney samples were collected for relative weight assessment and histopathological examination. **Results:** The results indicated dose-dependent histopathological alterations in tissues of both organs. The liver exhibited cellular degeneration, necrosis, and inflammatory infiltration, while the kidneys showed vascular congestion and glomerular hemorrhage, with the most pronounced damage observed at the higher exposure level. While average organ weights increased at both doses compared to the control group, those differences were not statistically significant. **Conclusion:** This study highlights the necessity to limit the use of synthetic food colorants and suggest their replacement with natural alternatives, particularly in food products intended for pediatric consumption.

Keywords: Allura Red, food colorant, liver, kidney, relative weight, histopathology

Introduction

In recent years, growing attention has been directed toward the potential health risks associated with synthetic food dyes. Azo dyes account for approximately more than 50% of the dyes manufactured annually worldwide (Chung, 2016). Allura Red AC (AR; FD&C Red No. 40, E129) is considered one of the most extensively utilized azo dyes. AR is incorporated into a variety of food products, pharmaceuticals, and cosmetics to enhance their visual appeal (Hashem *et al.*, 2010). Nevertheless, an increasing number of experimental studies suggested that the synthetic azo dyes cause serious health disorders such as genotoxicity and cytotoxicity, and has been linked to behavioral and neurological disorders (Stevenson *et al.*, 2010; Nigg *et al.*, 2012). Studies indicated also that AR is implicated in a variety of health disorders including anemia, allergic reactions, histopathological alterations, cancer, attention deficits, and behavioral disturbances (AsHIDA *et al.*, 2000; Helal & Abdel-Rahman, 2005; Moutinho *et al.*, 2007; Feng *et al.*, 2012; Vojdani & Vojdani, 2015; Khayyat *et al.*, 2018; Miller *et al.*, 2022).

Recent studies have documented systemic effects of AR beyond histopathology. Sharma *et al.* (2022) documented that AR exposure in Swiss albino male mice induced significant alterations in hematological, biochemical, and antioxidant parameters, including decreased antioxidant activity and elevated liver enzyme levels, indicating systemic toxicity at the given doses. Additionally, Hofseth *et al.* (2024) found that AR can induce DNA damage, inflammation, and gut microbiome disruption.

Studies by Khayyat *et al.* (2018) and Megahed *et al.* (2020) showed that animals treated with AR exhibited significant changes in liver and kidney biochemical markers and tissue structure such as elevated serum levels of bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein, albumin, globulin, creatinine, urea, and uric acid. Histopathological examinations displayed cytoplasmic vacuolization of hepatocytes, focal necrosis, and inflammatory cell infiltration in the liver, while renal tissues exhibited necrosis, tubular vacuolization,

glomerular destruction, and Bowman's capsule thickening. Alsolami (2017) observed that AR administration in rats induced noticeable alterations in hepatic biochemical parameters, including AST, ALT, alkaline phosphatase, glucose concentration, and total serum protein. Bawazir (2016) noticed that prolonged exposure to AR induced a marked increase in serum levels of creatinine, urea and renal tubular necrosis. Although several studies have investigated the general toxicological effects of Allura Red, limited information is available concerning its dose-dependent histopathological effects during sub-chronic exposure. Therefore, the present study was conducted to investigate the histopathological effects of a high dose of Allura Red AC on the liver and kidney of albino mice. This study aimed to provide a detailed descriptive assessment of tissue damage in those organs.

Materials and methods

Allura Red material

Allura Red (CAS 25956-17-6; 80% purity) was purchased from Enrico Giotti (Firenze, Italy).

Experimental animals

Thirty healthy male Swiss albino mice (19–24 g) were randomly assigned to three groups (n=10 each). Mice were housed under standard laboratory conditions with a controlled temperature of 22–25°C, relative humidity maintained at 50–60%, and a 12-hour light/dark cycle. All animals had unrestricted access to water and food. A one-week acclimatization period preceded the experiment (Reeves *et al.*, 1993). All procedures were carried out according to the Organization for Economic Co-operation and Development guidelines (OECD).

Experimental design and dosage

The animals were assigned into three experimental groups. Group I which given only the standard diet and served as controls, group II which treated with a low dose of Allura Red (700 mg/kg BW/day) along with the standard diet and group III which treated with a high dose of Allura Red (1400 mg/kg BW/day) along with the standard diet.

Each mouse exposed to Allura Red received a daily oral dose of 200 µL of the test solution for 42 days via gastric gavage. These doses were above the human Acceptable Daily Intake (0–7 mg/kg BW/day, as set by JECFA/WHO) to enable the identification of dose-dependent histopathological alterations under controlled experimental conditions. At the same time, they continued below the concentrations associated with acute toxicity or lethality (>5000 mg/kg BW, Additives & Food, 2009).

Relative weight analysis

At the end of the experiment, mice were euthanized and livers and kidneys were carefully removed and weighed using an analytical balance. Relative organ weights were quantified as a percentage of the final body weight for each animal (%BW) using the formula described by Xu *et al.* (2006).

Relative liver weight (%) = (Liver weight/Final body weight)×100

Relative kidney weight (%) = (Kidney weight/Final body weight)×100

For each organ, representative tissue samples were collected for histopathological examination.

Histopathological examination

For histopathological evaluation, three tissue sections were prepared from each organ of every animal. Liver and kidney samples were examined according to the method described by Bancroft and Gamble (2008). Briefly, tissues were fixed in 10% neutral-buffered formalin, then cut into small sections (≤10 mm×5 mm×3 mm) and processed using standard histological procedures, including dehydration, clearing, and paraffin embedding. Paraffin-embedded tissues were sectioned, mounted on glass slides, and stained with Hematoxylin and Eosin (H&E). The slides were then coverslipped using Mayer's adhesive and examined microscopically.

Statistical analysis

Data are expressed as mean±standard deviation (SD), with ten mice included in each group as biological replicates (n=10). All measurements were performed in triplicate to ensure technical accuracy, and the mean of these replicates was used for statistical analysis. A completely randomized design (CRD) was employed. One-way analysis of variance (ANOVA) was conducted using SAS software (version 2002) to detect differences among the three groups. Significant differences among groups were assessed using Duncan's multiple range test, with significance considered at $P<0.05$ (Steel *et al.*, 1980).

Ethical approval

This study was approved by the Bioethics Committee at the Libyan Center for Biotechnology Research (BEC-BTRC), under the reference No; NBC: 001.A.25.84.

Results

Changes in relative weight of studied organs

As illustrated in table 1, there were no statistically significant differences ($P>0.05$) in the relative weights of the livers and kidneys across all experimental groups. However, a slight increase in relative liver weight was observed in the treated groups, which may be attributed to hydropic degeneration in hepatocytes. Similarly, the relative kidney weights showed minor variations among treated groups; however, these alterations were not statistically significant.

Table 1. Effects of Allura Red on the relative weight of liver and kidney tissues of mice.

Groups	Relative liver weight (%)	Relative kidney weight (%)*
Control	4.75±0.22	1.85±0.17
Low dose (700 mg/kg BW/day)	[a]5.15±0.88	[b]1.99 ±0.34
High dose (1400 mg/kg BW/day)	[a]5.54±0.32	[b]1.98±0.13

Data represented as Mean±SD (n=10 each group). Each sample was examined in triplicate. [a,b] No significant differences to respective controls at the $P<0.05$ level. Relative weight of liver and kidneys of albino mice is based on 3 replicates (n=3). BW=body weight. *Total weight of the right and left kidneys.

Histopathological effects of Allura Red

In comparison with the control group, mice treated with Allura Red showed dose-dependent histopathological changes in both liver and kidney tissues. Moreover, a significant increase in lesions was revealed in the high-dose group compared with the low-dose and control groups.

- ***Histopathological hepatic changes***

Microscopic analysis revealed a clear dose-dependent pattern of hepatic tissue damage. The low-dose group showed moderate dilation and congestion of the central vein, focal necrosis, and infiltration of inflammatory cells (Figure 1A–C). In contrast, the high-dose group revealed more pronounced congestion of the central vein, marked hydropic degeneration of hepatocytes, and evident apoptotic cell death, reflecting considerable hepatic damage (Figure 2A–B).

- ***Histopathological renal changes***

Similar to those of liver, kidney tissues exhibited dose-dependent pathological alterations. The low dose group showed mild dilation and congestion of the renal blood vessels (Figure 3A). In contrast, the high-dose group displayed severe histopathological features, such as focal hemorrhage, vascular congestion, and glomerular bleeding, indicating significant renal injury (Figure 3B–C).

Discussion

The current findings indicate that Allura Red induces dose-dependent histopathological alterations in both hepatic and renal tissues, with the most noticeable effects were observed at higher exposure levels. In addition, there were no significant differences in the relative weights of these organs compared to controls, although higher liver weights were noted. These findings are consistent with those of El-Wahab and Moram (2013) and Megahed *et al*. (2020), who reported that azo dyes did not cause significant changes in relative liver or kidney weights in rats, with the exception for Brilliant Blue.

On other hand, Raya *et al*. (2020) reported a significant increase in relative liver and kidney weights in rats administered a low dose of Allura Red. Conversely, Reza *et al*. (2019) showed a significant decrease in relative weights of liver and kidneys in mice treated with an azo colorant; Carmoisine. The results from this study contradicted the findings of Fahmy *et al.* (2021) who reported that liver and kidney weights significantly increased following treatment with a high dose of Sunset and low and high doses of a mixture of Tartrazine and Brilliant blue.

Regarding the histopathological analysis, our observations are consistent with the findings of Megahed *et al.* (2020), who demonstrated that dietary exposure to Allura Red (400 mg/kg for 60 days) produceed marked hepatic and renal alterations, including hepatocyte necrosis, hydropic degeneration, vacuolization, sinusoidal congestion, and leukocyte infiltration. Moreover, our findings are in agreement with Khayyat *et al.* (2018) who documented histopathological damage in the liver and kidneys of albino rats following oral administration of Allura Red (7 mg/kg BW for 4 weeks). The liver exhibited vascular congestion, fibrosis, and necrosis, while renal tissues showed tubular degeneration and pyknotic nuclei. The results are also in agreement with previous study by Khayyat *et al.*, (2017), who reported liver and kidney structural damages in rats following oral administration of the azo food colorant; Tartrazine. Additionally, Bawazir (2016) reported nephrotoxic effects in mice treated with Allura Red at 100 and 200 mg/kg BW/day for 56 days, including glomerular atrophy, necrosis, and deformation of renal tubules, associated with enlarged hepatocytes.

However, the present results are different from those of Tsuda *et al.* (2001), who found no histopathological alterations in liver or kidney tissues of mice treated with Allura Red at 700 mg/kg BW.

The observed tissue damage following exposure to Allura Red could be attributed to oxidative stress–induced toxicity. Azo dyes such as Allura Red are degraded into reactive intermediates that enhance the excessive generation of reactive oxygen species (ROS), leading to lipid peroxidation, protein oxidation, and mitochondrial dysfunction in hepatic and renal cells (Amin *et al.*, 2010; Khayyat *et al.*, 2018; Sharma *et al.*, 2022). Increased oxidative stress can activate cyclooxygenase-2 (COX-2) expression, promoting the production of pro inflammatory mediators, contributing to vascular congestion, and inflammatory infiltration. This may elucidate the central vein congestion in the liver and the vascular disturbances observed in the kidney (Khayyat *et al.*, 2018). Moreover, oxidative imbalance may disrupt the regulation of programmed cell death by down regulation of the expression of the anti-apoptotic protein Bcl-2, leading to increased cellular susceptibility to apoptosis and necrosis. This mechanism may provide the possible explanation of hepatocellular degeneration, hydropic changes, and apoptotic cell death detected especially in the high dose group (Khayyat *et al.*, 2018; Sharma *et al.*, 2022). The dose-dependent liver and kidney lesions suggests that higher concentrations of Allura Red may exceed the capacity of endogenous antioxidant defense mechanisms, resulting in pronounced structural and functional impairment of these tissues.

The doses used (700 and 1400 mg/kg BW/day) were based on the documented subchronic no-observed-adverse-effect level (NOAEL) of 695 mg/kg BW/day in rodents (Borzelleca *et al.*, 1991; Tanaka, 1994). The lower dose represented the toxicity threshold, while the higher dose exceeded NOAEL to evaluate dose-dependent toxicity. Both doses remained below acute lethal concentrations (EFSA, 2009) and were chosen to investigate hepatic and renal histopathological effects and to characterize potential health risks at high-exposure levels. Extrapolation to humans using standard safety factors indicates that typical dietary exposure is likely safe, whereas the effects observed in rodents emphasized potential risks under high-exposure.

A limitation of the present study is that semi-quantitative scoring of tissue lesions was not performed, as the study was primarily designed as a descriptive histopathological investigation aimed at identifying dose-dependent structural alterations in hepatic and renal tissues. Furthermore, no standardized grading system has been

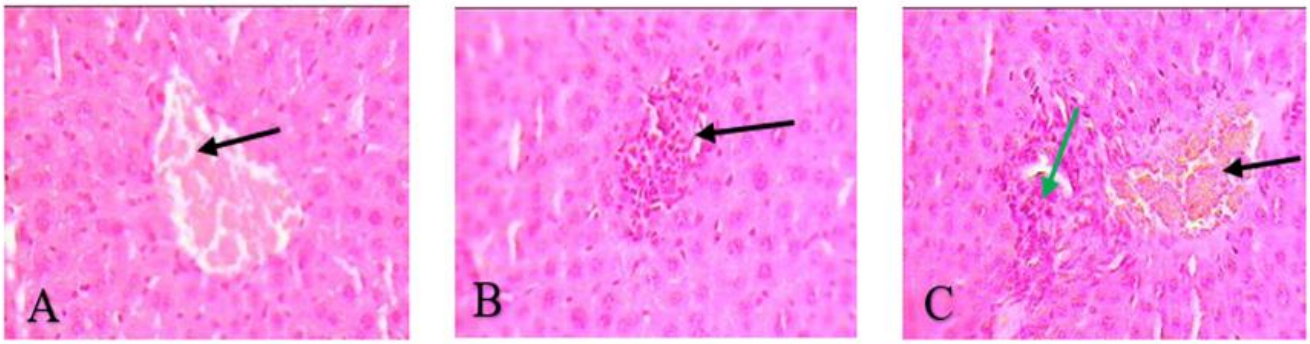

Figure 1. Histological alterations in the liver tissues of mice administered a low dose of Allura Red (700mg/kg BW/day). H&E stain (x400). A. Central vein dilation and congestion (black arrow). **B.** Necrosis of part of the liver cells accompanied by infiltration with inflammatory cells (black arrow). **C.** Congestion in the central vein (black arrow) and necrosis of liver cells accompanied by infiltration of inflammatory cells (green arrow).

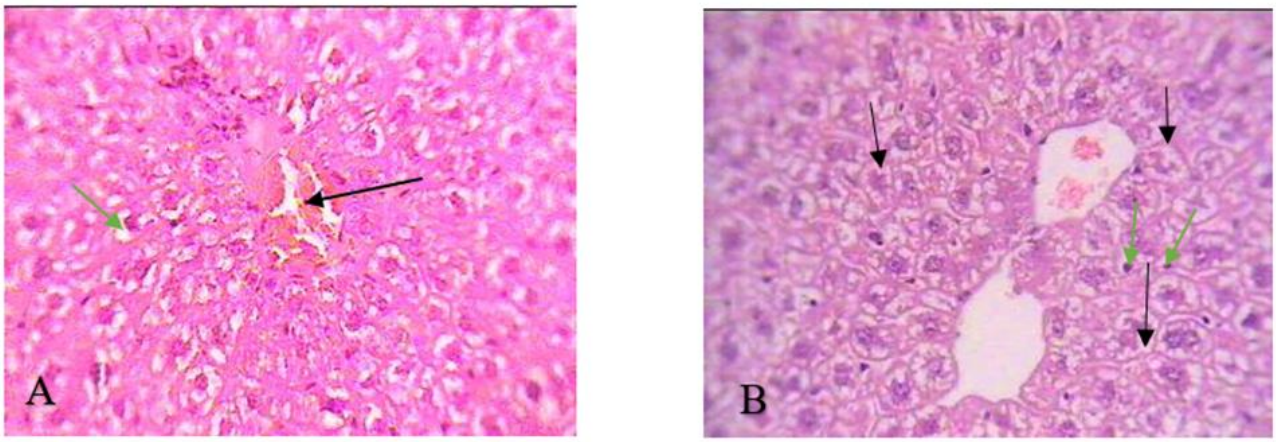

Figure 2. Adverse effect of Allura Red in the liver of mice following the high dose treatment (1400mg/kg BW/day). H&E stain (x400). A. Congestion in the central vein (black arrow) and hydropic degeneration in liver cells (green arrow). **B.** Hydropic degeneration in liver cells (black arrow) and apoptosis of some cells (green arrow).

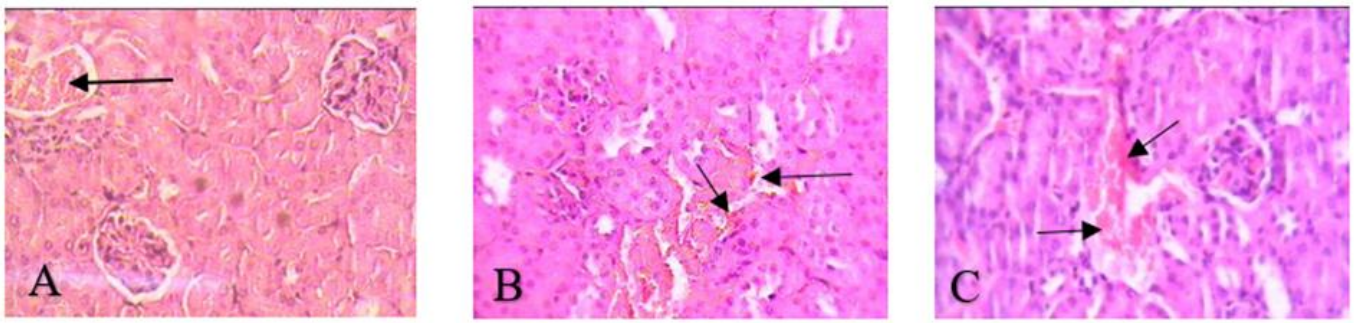

Figure 3. Allura Red alters the normal histology of the kidney of mice following treatment. H&E stain (x400). A. Dilatation and congestion of the renal blood vessels (black arrow) following low dose (700 mg/kg BW/day) treatment. **B.** Localized hemorrhage (black arrow) following high dose (1400 mg/kg BW/day) treatment. **C.** Congestion and glomerular hemorrhage (black arrow) following high dose (1400 mg/kg BW/day) treatment.

specifically established for Allura Red–induced lesions in this experimental model (Hofseth *et al.*, 2024).

Conclusion

The synthetic azo dye administered in this study caused pronounced histopathological changes in the liver and kidneys of mice. These findings highlight potential health risks for humans and emphasize the importance of minimizing the use of such food colorants, particularly in products intended for children. Additionally, the type and concentration of colorants in food products should be clearly labeled. Future studies should employ blinded semi-quantitative scoring and complementary biochemical analyses to provide a more comprehensive assessment of damage severity.

Author contributions

Hana E. Inbaya designed the study. Otman N. Ermithi bought the Allura red. Hana E. Inbaya, Shadia G. Ramadan, Amal O. Buker and Abuajila A. Tarhuni interpreted the results. Shadia G. Ramadan wrote the manuscript. All authors read, reviewed and approved the final version of the manuscript.

Conflict of interest

The authors declare no conflict of interest in relation to the publication of this work.

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