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Evaluation of the Protective and Immunomodulatory Efficacy of *Annona Muricata* Leaf Extract Against Allura Red (E129)-Induced Nephrotoxicity and Inflammation in Male Albino Rats

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Abstract

Background: Synthetic food colorants, including Allura Red (E129), are extensively utilized in ultra-processed foods and have been implicated in inducing oxidative stress, cellular damage, and inflammatory responses. **Objective:** This study aimed to evaluate the protective and immunomodulatory efficacy of an aqueous extract of *Annona muricata* leaves against Allura Red (E129)-induced nephrotoxicity and inflammation in male albino rats. **Methodology:** An experimental study was conducted at the animal house of the College of Pharmacy, University of Kerbala, Iraq from November 2025 to December 2025. The aqueous extract of *Annona muricata* leaves was prepared at Al-Ameed University. Twenty adult male albino rats were randomly assigned to four groups (n=5 per group) and subjected to their respective treatments for a duration of 30 days. **Results:** Oral administration of Allura Red E129 alone (Group 2) induced severe nephrotoxicity, evidenced by a significant depletion in serum GSH levels (47.97 ± 1.83) and a marked elevation in MDA levels (37.67 ± 1.06). These biochemical alterations were histopathologically accompanied by glomerular congestion, obliteration of Bowman's spaces, and acute tubular necrosis. Conversely, pre-treatment with *A. muricata* leaf extract (Group 4) significantly mitigated these damaging effects, restoring GSH levels to 97.92 ± 1.70 and reducing MDA levels to 14.95 ± 0.17. **Conclusion:** In conclusion, the aqueous leaf extract of *Annona muricata* exerts potent antioxidant and defensive activities, efficiently suppressing the immune-inflammatory cascades and histopathological alterations induced by Allura Red E129. These findings highlight its therapeutic potential in preserving renal structural and functional integrity. *[Bangladesh Journal of Infectious Diseases, June 2026;13(1):160-165]*

Keywords: *Annona muricata*; Histopathology; Kidney; Allura red E129; Immunomodulation

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Introduction

Though having a long history, treatment by medicinal plants is still among the main ways of treating different illnesses of people and animals belonging to various ethnic groups in different countries. Increasing interest in herbal medicines is caused by fewer side effects that these drugs demonstrate on biological surroundings of the organism and on non-specific cell[1,2]. Plants and their biologically active compounds are the subjects of increasing interest because of their potential to be used as new pharmacological agents[3].

Various forms of drugs have been applied to prevent and cure illnesses and improve health state of humans and animals. One of such drugs is soursop (Annona muricata)[4]. Soursop (*Annona muricata*),

also called graviola, is one of the plants attracting particular attention because of its therapeutic potential. Different parts of the plant are used by tropical population in traditional therapy; leaf, stem bark, root, and seeds are considered to be the main medicinal constituents. Leaves of *Annona muricata* are known to have anticancer, antioxidant, and antibacterial properties[5-6].

They contain not only essential oils but also acetogenins, alkaloids, flavonoids, and other biologically active substances making them suitable for medical purposes[7]. Beverages prepared from the dried leaves of *A. muricata* and decoctions made from fresh leaves are used to treat chronic diarrhea, dysentery, hypertension, kidney, and bile disorders and have anticancer activity. The biological effects of drinks and extracts of *A. muricata* leaves are based on their content of biologically active components such as phenols, alkaloids, and acetogenins. Among phenols found in *A. muricata* leaves, phenolic acids, flavonoids, and gallotannins should be mentioned. Alkaloids found in leaves and stem barks of *A. muricata* are aporphines, isoquinolines, imine sugars, and protoberberines. As for acetogenins, at least forty compounds were isolated from its leaves, with annonacin being the most abundant one[8-9].

Synthetic colorants became rather common in various branches of industry, especially food and pharmaceutical industries, as a way of industrial marketing. Consuming synthetic dye, Allura Red, which was added to food, can pose certain dangers to the human body, causing such disorders as genotoxicity[7], cancer, attention deficit hyperactivity disorder (ADHD), allergy, and asthma in children[10,11]. Allura Red AC (E129; Food D&C Red No. 40) is an artificial coloring used in the manufacture of ultra-processed food products, hence found commonly in foodstuffs like sodas, confectionery, ice creams, and baked goods[12]. The dye is linked to changes in oxidative stress, neurotransmission, mitochondrial metabolism, inflammation, and kidney damage[9]. It is known to significantly affect brain physiology and general neural health and has links to allergies, food intolerance, cancers, multiple sclerosis, attention deficit hyperactivity disorder (ADHD)[13], brain damage, nausea, heart diseases, and asthma. A number of nations have banned the use of the Allura Red coloring in foods and beverages and have put tight regulations on its application[14].

This study was aimed to investigate the protective and immunomodulatory efficacy of an aqueous extract of Annona muricata leaves against Allura Red (E129)-induced nephrotoxicity and inflammation in male albino rats

Methodology

Study Settings: This experimental study was conducted at the animal house of the College of Pharmacy, University of Kerbala, Iraq from November 2025 to December 2025. The study population consisted of twenty (20) adult male white albino rats, weighing between 250 to 320 grams and aged 12 to 14 weeks.

Selection Criteria: Healthy, active rats within the specified weight and age range were included in the study, while any animals demonstrating pre-existing signs of illness or physical injury during the initial two-week acclimatization period were excluded. The selected animals were housed in clean plastic cages with wire mesh tops under controlled laboratory conditions, which included a constant temperature of 25°C and a structured 12-hour light/12-hour dark cycle. Standard laboratory chow and water were provided and libitum throughout the experimental period. Following acclimation, the twenty (20) rats were systematically divided into four equal groups (n = 5 rats per group) and treated for 30 days as follows:

Negative Control Group (G1): Animals were administered daily orally with normal water solution. Positive Control Group (G2): Animals were orally administered E129 red dye at a concentration of 0.4 mg/kg body weight every other day. Group 3 (G3): Animals were orally administered daily an aqueous extract of soursop (*Annona muricata*) leaves at a concentration of 400 mg/kg body weight Group 4 (G4): Animals in this group were orally administered daily an aqueous extract of soursop (Annona muricata) leaves at a concentration of 400 mg/kg body weight, 4 hours before being orally administered E129 red dye at a concentration of 0.4 mg/kg every other day.

Plant Used: The leaves of soursop (*Annona muricata*) were procured from a specialized herbal shop in Baghdad, Iraq. The plant material was officially authenticated and classified by Prof. Dr. Nibal Al-Mutairi, a specialist in plant taxonomy at the Department of Biology, College of Education for Pure Sciences, University of Kerbala. The subsequent preparation of the aqueous leaf extract was conducted at the laboratories of Al-Ameed University.

Preparation of the Aqueous Extract: The leaves of the soursop plant were picked and washed extensively using distilled water to remove all

external impurities like dust and dirt that might contaminate the leaves during the extraction procedure. The washing process is important since it ensures that there is no contamination of the bioactive compounds during the extraction. Once the leaves had been washed, they were allowed to dry naturally under normal room temperature conditions. The aim here is to dry the leaves naturally without exposing them to heat because high heat can destroy the bioactive compounds. The dried leaves were ground finely using the electrical grinder to increase the contact area of the leaf matter.

Collection of Aqueous Extracts: Aqueous extracts of the leaves were obtained through soaking 10 to 50 g of powdered leaves in 100 to 500 mL of distilled water with gentle stirring at 40° to 45°C inside the oven. The obtained mixture was subjected to a sterilization process by pressurized steam sterilization in a steam autoclave. This technique makes use of high-pressure saturated steam at elevated temperatures to ensure complete extraction of biologically active substances due to cell wall lysis in plants and their release into the liquid medium. The liquid was later filtered first using multi-layered medical gauze and then filtered using the precision filter paper to obtain the clear filtrate solution, which was stored at 4°C before undergoing further analysis[5].

Anesthesia of Rats: Anesthesia was induced in the animal through the use of chloroform before the dissection procedure began, during which the abdominal wall of the animal was cut with scissors and a scalpel, after which the kidney was removed and cleaned.

Preparation of Histological Sections: Following this, for preservation purposes, the animal organs were dissected along the longitudinal and transverse planes in smaller pieces. After soaking for 48 hours in 10.0% formalin solution, the specimens were dehydrated by means of rising levels of alcohol solutions. Clearing was done using xylene before infiltration with paraffin wax in an oven set at 56°C to make molds. All the tissues were then stained using hematoxylin and eosin following the preparation of sections with a 5 μm thickness by the microtome.

Statistical Analysis: The statistics involved in analyzing the results were done using the SAS program version 9. The results will be reported as mean ± standard error (Mean ± SE). The data from the tables have been analyzed using t-test, ANOVA, and LSD test at 0.05 level of significance[12].

Ethical approval: The present study received formal ethical clearance from the Institutional Scientific and Research Ethics Committee under protocol number 268 on 16 September 2025. All protocols involving animal subjects complied fully with globally accepted benchmarks for the humane treatment and care of laboratory animals. Every effort was directed toward mitigating animal suffering, minimizing sample sizes, and maintaining optimal environmental and veterinary conditions throughout the experimental timeline. Additionally, the establishment of the autism-like model using valproic acid, as well as the related behavioral and histopathological analyses, strictly followed the approved experimental design.

Results

Microscopic analysis of the kidney sections obtained from the experimental animals revealed distinct histopathological variations across the different groups (Figure I).

Negative Control Group (G1): Examination of the renal tissue in this group showed standard architecture with no structural deviations. The morphology of Bowman's capsules, proximal convoluted tubules, distal renal tubules, glomeruli, and the renal cortex appeared entirely healthy and intact (Figure IA).

Positive Control Group (G2): In contrast, the group administered Allura Red E129 alone (0.4mg/kg) exhibited severe histopathological alterations in the kidney tissue. These changes were characterized by significant glomerular congestion,

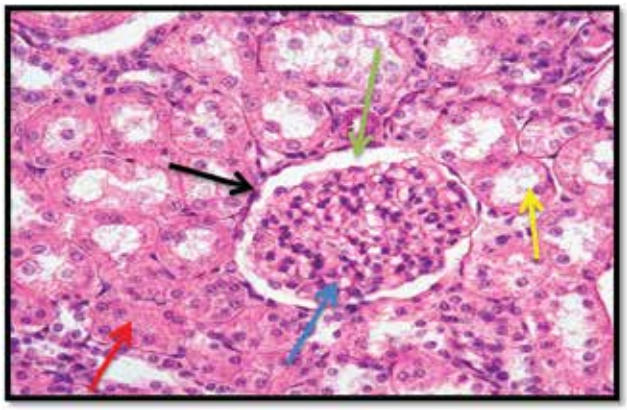

Figure I: (control group) showing the light microscopic appearance of a histological slice of normal kidney. Here, the glomerulus can be seen using the blue arrow and is surrounded by Bowman's capsule (black arrow). Glomerular vacuoles can be observed by the green arrow, while the proximal convoluted tubule and distal convoluted tubule are marked by the red and yellow arrows, respectively.

blocked Bowman's spaces, marked renal tubule damage, and acute tubular hydronephrosis consistent with acute tubular necrosis (Figure IB).

Soursop Extract Group (G3): Renal sections from the rats treated solely with the aqueous extract of Annona muricata leaves (400 mg/kg) demonstrated well-preserved renal architecture. The structure of Bowman's capsules, distal tubules, glomeruli, and proximal convoluted tubules stained with hematoxylin-eosin appeared normal, showing no noticeable abnormalities in comparison with the control samples (Figure IC).

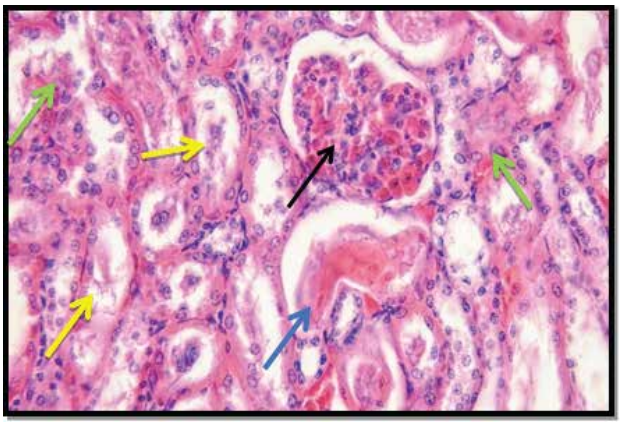

Figure II: Kidney from a Rat. Allura Red E129 injection at 0.4 mg/kg produced marked changes histopathologically involving the glomerulonephritis with blocked Bowman's spaces (black arrow), renal tubules (blue arrow), hydronephrosis (yellow arrow), and acute tubular necrosis (green arrow)

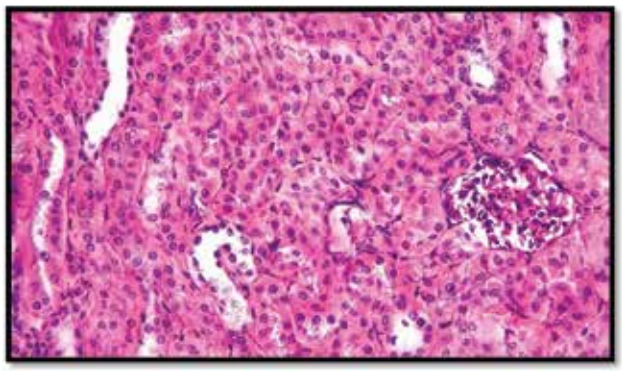

Figure III: Showing the microscopic image of kidney tissue samples obtained from rats in the 400 mg/kg extract treatment group. The micrograph shows the glomerulus (indicated with blue arrow), which is surrounded by Bowman's capsule (indicated with black arrow), glomerular vacuole (indicated with green arrow), proximal convoluted tubules (indicated with red arrow), and distal convoluted tubules (indicated with yellow arrow)

Protective Group (G4): For the group pre-treated with A. muricata leaf extract (400mg/kg) four hours prior to Allura Red E129 administration, the plant extract significantly mitigated the toxic dye-induced damaging effects. The tissue showed only minor histological variations, represented by a mild enlargement of Bowman's spaces, alongside relatively healthy glomeruli and tubules maintaining a normal overall morphology (Figure ID). Compared to the severe damage observed in the positive control (G2), these outcomes clearly imply that the proximal and distal tubules experienced only limited, regenerative degenerative histology in a few isolated cells (Figure 1D).

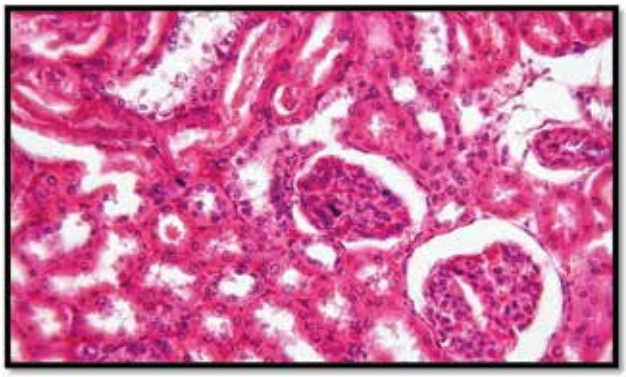

Figure IV: Showing an image of a mouse kidney tissue after exposure to Annona muricata extract at a dose of 400 mg/kg and stained by Allura Red E129 at 0.4 mg/kg. Organs found in the figure include glomerulus (blue arrow), Bowman's capsule (black arrow), glomerular vacuole (green arrow), proximal convoluted tubule (red arrow), and distal convoluted tubule (yellow arrow)

The effects of Annona muricata leaf aqueous extract (soursop) on serum GSH and MDA levels in healthy adult albino rats dosed with Allura red E129. Letters in uppercase that appear in a vertical order show statistically significant difference ($p \leq 0.05$) (Table 1).

The biochemical parameters presented in Table 1 demonstrated significant alterations among the experimental groups. Oral administration of Allura Red E129 alone (Group 2) induced severe oxidative stress, as evidenced by a substantial depletion in serum GSH levels to 47.97 ± 1.83 compared to the control group (108.27 ± 1.10), accompanied by a marked elevation in MDA levels to 37.67 ± 1.06. Conversely, rats treated with Annona muricata extract alone (Group 3) exhibited a significant enhancement in antioxidant capacity, with GSH levels rising to 148.44 ± 1.33 and MDA levels decreasing to 8.89 ± 0.20. Notably, pre-treatment

with *A. muricata* extract prior to Allura Red administration (Group 4) effectively mitigated the induced oxidative damage, successfully restoring GSH levels toward normal values (97.92 ± 1.70) and significantly suppressing MDA accumulation to 14.95 ± 0.17.

Table1: Biochemical Parameters Demonstrated Significant Alterations among the Experimental Groups

Treatment	Means ± stderr	
	GSH	MDA
G1 control	108.3 ±1.10 B	13.8 ± 0.95 B
G2 Allura red E129 0.4mg	48.0 ±1.83 D	37.7 ± 1.06 A
G3 *Annona muricata* 400mg/kg	148.4 ±1.33 A	8.9 ± 0.20 C
G4 *Annona muricata* 400mg/kg+ Allura red E129 0.4mg	97.9 ± 1.70 C	14.9 ± 0.17 B
L.S.D	**4.5695**	**2.1784**

Discussion

An analysis of the protective effects of the aqueous leaf extract of Annona muricata (soursop) against the serum antioxidant profile characterized by malondialdehyde (MDA) and glutathione (GSH) in healthy white rats after exposure to Allura Red E129 have been performed. There is a statistically significant decline in GSH ($P \leq 0.05$) and the presence of a significant increase in MDA in the Allura Red–exposed groups. Particularly, group G2 was made up of white rats given Allura Red E129 at a dosage of 0.4 mg/kg body weight each day for 30 days (positive control) compared to group G1 (negative control), with the GSH value at 47.97 ± 1.83 while MDA value was 37.67 ± 1.06, respectively. Group G3 recorded a significant increase in the level of GSH (148.44 ± 1.33) and a significant decrease in the level of MDA (8.89 ± 0.20) as compared to the negative control (G1). Also, group G4 had a significant increase in the level of GSH and a significant decrease in the level of MDA compared to group G2, with the values of GSH and MDA being 97.92 ± 1.70 and 14.95 ± 0.17, respectively.

This is in agreement with the findings of Amin et al15 where there was a decrease in GSH and MDA levels in male rats after treatment with Allura Red E129 at 50 mg/kg daily for 10 days. There was further confirmation from another study conducted where white rats were administered with Allura Red E129 orally at 7 mg/kg in four weeks resulting in a significantly increased MDA and a decrease in GSH level. Glutathione is a tripeptide made up of three amino acids and functions as an important antioxidant in many organisms[15].

Glutathione is recognized as the key nonenzymatic antioxidant that neutralizes free radicals and the products thereof. Accordingly, oxidative stress causes free radicals production and, therefore, increased use of glutathione[16]. Lipid peroxidation can damage cell membranes due to oxidative stress. Superoxide anion (O2−) reacts with lipids to produce lipid peroxide; then, lipid peroxide undergoes beta oxidation and converts into malondialdehyde (MDA)[17]. The phytochemical analysis of *Annona muricata* (soursop) leaf extracts revealed several chemical compound types such as alkaloids, flavonoids, terpenoids, coumarin and lactones, anthraquinones, tannins, cardiac glycosides, phenols, phytosterols, and saponins. These compounds were found in ethanolic and aqueous extracts of the leaves with a different degree of intensity from low to high[18,19].

It should be noted that the antioxidant activity of the soursop leaf extract was relatively higher compared to that of other compounds studied. Compounds contained in the soursop fruits' extract, such as flavonoids, terpenoids, phenols, steroids, and alkaloids, can reduce the levels of MDA and raise the level of glutathione (GSH). Phenols and flavonoids are commonly used as key components responsible for the antioxidant effect of medicinal plants[20]. Antioxidants and inhibitors of lipid peroxidation can scavenge free radicals and protecting liver and kidney cells. This effect is achieved owing to an increased amount of antioxidant constituents in the soursop fruits' leaf extract, particularly, phenols, unsaturated fatty acids (for example, gallic acid, benzoic acid, salicylic acid), and numerous esters, alkaloids, and cyclopeptides[21].

Conclusion

From these results, it is clear that aqueous leaf extract of Annona muricata possesses potent antioxidant activity, produces positive effects on kidney parameters of male rats, and mitigates toxic actions and histopathological changes caused by Allura red (E129).

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None

Conflict of interest:
The Author has declared no conflict of interest in this work.

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Authors' contributions
The whole work was done by Mohammed Yahya Al-Yasari, under the supervision of Dr. Muhammed W. H. Al-Muhanna. Both authors approved the final manuscript.

Data Availability
Any questions regarding the availability of the study's supporting data should be addressed to the corresponding author, who can provide it upon justifiable request.

Ethics Approval and Consent to Participate
All experimental procedures for animals were conducted in accordance with approved ethical controls and after obtaining approval from the Institutional Animal Care and Use Committee (IACUC) at the University of Karbala - College of Veterinary Medicine, under approval number: UOK.VET.BI.2026.177, in full compliance with the animal welfare standards applicable in experimental studies.

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