Evaluation of the protective role of the aqueous extract of soursop (*Annona muricata*) leaves against hepatotoxicity and physiological changes induced by Allura red (E129) dye in male albino rats

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***Abstract*—** This work aimed to identify the biological agent, in the aqueous extract of soursop leaves (*Annona muricata*), to identifying oxidative stress induced by Allura red E129, using the latter as a model. For 30 days, rats were divided into four groups. Group 2 received two weekly orally administered of Allura red at a dose of 0.4 mg/kg body weight, unlike Group 1, which served as the control group. Group 3 received an aqueous extract of soursop leaves at a concentration of 400 mg/kg.Group 4 received an aqueous extract of soursop leaves at a concentration of 400 mg/kg daily, four hours before receiving Allura red E129. Results: The results of this study indicate that A. muricata is also effective in protecting against the pathogens associated with Allura red E129, as evidenced by the associated degenerative cell expression, congestion, glomerular shrinkage, and Bowman's absence. By decrease ALT, AST and ALP, a 400 mg/kg aqueous extract of A. muricata was observed to significantly reduce the effects of Allura red in rats. Furthermore, the extract demonstrated enhanced oxidative stress defense against toxicity from A. muricata L. golders, exhibiting antioxidant activity.

Keywords*:* *Annona muricata*, Histopathology, Liver, Allura red E129.

INTRODUCTION

Medicinal plants are a promising source for the traditional raw herbal medicine industry, which uses them to reduce, treat, or control the risk of disease, due to their potent antioxidant and anti-inflammatory effects (1). Humans have used various types of medicines to treat and prevent diseases and promote human and animal health. One plant used to treat many ailments is soursop (*Annona muricata*) (2).

Soursop, also known as *Annona muricata*, has garnered significant attention for its therapeutic potential. All parts of the plant are used in traditional medicine by people in tropical regions, with the leaves, stem bark, roots and seeds being the primary medicinal components (3). Soursop leaves (*Annona muricata*) are known to have anticancer, antioxidant, and antibacterial effects. In addition to essential oils, these leaves contain high levels of acetogenins, alkaloids, flavonoids, and other chemical compounds that make them beneficial in medicine (4).

Thanks to its pharmaceutical applications, the leaves of the soursop plant contain a wide range of phytochemicals identified in various studies. Some of these are limited to phenolic compounds found in plants cultivated in specific regions, in contrast indicate anti-angiogenic compounds through metabolic analysis or as a source of antioxidant compounds (5).

Drinks made from dried leaves and decoctions of fresh leaves are used as remedies for chronic diarrhea, dysentery, hypertension, and kidney and biliary tract diseases. They also possess anticancer properties. The biological activities of drinks, decoctions, or extracts of Annon muricata leaves are linked to their content of bioactive compounds such as phenols, alkaloids, and acetogenins. Some specific phenolic compounds include phenolic acids, flavonoids, and gallotannins. The leaves and bark of A. muricata also contain alkaloids such as aporphins, isoquinolines, imine sugars, and protoberiberins. Regarding acetogenins, more than forty compounds have been isolated from its leaves, with annonacin being the most abundant (6).

Synthetic dyes are becoming increasingly widespread, particularly in food and pharmaceuticals, as an industrial marketing strategy to attract consumers. However, the consumption of the synthetic azo dye Allura Red poses potential risks to human health and can cause several adverse health effects, including genotoxicity, cancer, attention deficit hyperactivity disorder (ADHD), allergies, and asthma in children (7).

Allura Red AC (also known as E129) FD&C (Red No. (40)) is an artificial food coloring widely used in the manufacture of ultra-processed foods, and is therefore commonly used in our food sources such as soft drinks, sweets, ice cream and bakery products (8). This coloring affects oxidative stress, neurotransmitter imbalances, mitochondrial dysfunction, inflammatory responses and the occurrence of kidney damage (9). It can significantly affect brain function and overall neurological health, including allergies, food intolerances, cancer, multiple sclerosis, attention deficit hyperactivity disorder, brain damage, nausea, heart disease and asthma (10). Many countries have banned the use of Allura Red coloring in food and beverage products and imposed strict controls on it (11).

MATERIALS AND MTHODS

Experimental Animals in the Present Study

In this study, 20 adult male white rats, weighing between (250-320) grams and aged between (12-14) weeks, were used. They were raised in the animal house of the College of Pharmacy / University of Karbala, from mid-November 2025 to mid-December 2025. The animals were placed in special plastic cages covered with metal lids. The floor was covered with fine wood shavings, and care was taken to keep the cages clean, changing the floor continuously and disinfecting it with disinfectants. Continuous care was also taken to keep the water bottles and the housing room clean. All experimental animals were subjected to suitable laboratory conditions in terms of temperature of 25 degrees Celsius, lighting duration (12 hours light - 12 hours darkness), and ventilation. The animals were provided with water and standard feed freely (Ad libitum) throughout the research period. The animals were left for two weeks to adapt to the conditions before the experiment and to ensure that they were free of diseases.

The Plant Used

The leaves of the prickly custard apple plant were obtained from a private herb shop in Alwa Al-Rashid, Baghdad, and classified in the Department of Life Sciences, College of Education for Pure Sciences, University of Karbala, by Professor Nibal Amteirrad, specializing in plant taxonomy. The aqueous extract was prepared in the laboratories of Al-Ameed University.

Preparation of the Aqueous Extract

The leaves of the soursop tree were collected and then thoroughly washed with distilled water to remove any external impurities such as dust, dirt, and other potential contaminants. This meticulous washing process is essential to prevent any contamination that could affect the extraction and analysis of the bioactive compounds. After washing, the leaves were dried naturally at room temperature. This method was chosen to preserve the integrity of the bioactive compounds; as excessive heat could damage these delicate molecules. The drying process continued until the leaves were completely dry. The dried leaves were then ground into a fine powder using an electric grinder. The grinding process increases the surface area of the plant material, thus improving the efficiency of the extraction process.

The aqueous extract of the plant was prepared by soaking 10–50 grams of the leaf powder in 100–500 ml of distilled water with continuous stirring at a temperature of 40–45°C in an oven. This mixture was then sterilized in a steam autoclave. Pressurized steam sterilization, which uses high-pressure saturated steam at elevated temperatures, ensures thorough extraction of the biologically active components from plant material. The high temperature and pressure facilitate the lysis of plant cell walls, releasing phytochemicals into the solution.

After steam sterilization, the solution was first filtered through several layers of medical gauze and then through high-precision filter paper. The clear filtrate was then stored at 4°C to maintain its stability and prevent microbial growth before further examination and analysis. This preparation method was used (4).

Experimental Design

The study comprised two experiments. The first experiment involved determining the most effective aqueous extract concentration from among three ascending concentrations (200, 300, and 400 mg/kg) considered safe for use.

The second experiment evaluated the effectiveness of the aqueous extract at the concentration selected based on the results of the first experiment (400 mg/kg) against toxicity. Twenty male albino rats were used in this experiment and systematically divided into four groups of five rats each, as follows:

1. Negative Control Group (G1): Animals were administered daily orally with normal water solution for 30 days.
2. Positive Control Group (G2): Animals were orally administered E129 red dye at a concentration of 0.4 mg/kg body weight every other day for 30 days.
3. Group 3 (G3): Animals were orally administered daily an aqueous extract of soursop leaves at a concentration of 400 mg/kg body weight for 30 days.
4. Group 4 (G4): Animals in this group were orally administered daily an aqueous extract of soursop 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 for 30 days.

Anatomy of the Animals to Collect Samples

The animals were anesthetized by using a cotton swab with chloroform, and placed in a sealed, transparent container. The animal was then quickly placed inside, and the lid was resealed. After confirming that the animal was anesthetized, it was removed, and blood was drawn directly from the heart via a 5 ml sterile syringe to obtain the maximum blood volume. The blood samples were immediately placed in sterile, anticoagulant-free gel tubes. The tubes were then centrifuged at 3000 rpm for 15 minutes to obtain the serum, which was transferred to small, clean, dry, labeled Eppendorf tubes. The serum was stored in a refrigerator at -20°C until biochemical tests were performed. These tests included liver function tests (AST, ALT, and ALP).

Tissue Sampling

After drawing blood from the male rats, they were dissected directly by making an incision in the abdominal cavity from

below towards the heart. The liver and kidneys were removed after removing the fatty tissue and the surrounding connective tissue. They were then washed with distilled water to remove the blood from them. After that, they were dried by placing them on filter paper. These organs were cut into small pieces longitudinally and transversely for ease of preservation while ensuring that the preservative reached them. The organs were then preserved in 10% formalin in clean plastic containers after labeling them and sealing them tightly, for 48 hours until the histological preparations were carried out on them. All tissue sections were then stained with hematoxylin and eosin after molding and section placement in a rotating microtome at 5 µm (12,13).

STATISTICAL ANALYSIS

Analysis of Variance (ANOVA) was used to examine the data in one direction and track the least significant adjusted differences (LSD), expressed as mean ± standard deviation. Results were considered statistically significant at $p < 0.05$. (14).

RESULT AND DISCUSSION

The Histological study: the control group in Fig. 1. In accordance with Shivakumar et.al., (15). Photomicrograph of a liver section shows histologically normal tissue. The tissue maintains its normal architectural structure. The hepatocytes are arranged in radial cords (black arrow). The hepatic sinusoids are clearly visible as thin, light-colored spaces between the cords (red arrows). There are several small, dark areas, often representing the portal areas/portal triads (blue arrow) and central vein (green arrow) (H&E stain, 100X). hepatic histological examination that is related to the positive control group (G2) Fig. 2 Animals were orally administered E129 red dye at a concentration of 0.4 mg/kg body weight every other day for 30 days: Photomicrograph of a liver section. The section shows significant Hepatocyte (blue arrow), showing evidence of cytoplasmic vacuolar degeneration and micro-vesicular steatosis (black arrows). Binucleated cells are visible result of necrosis (red arrows) Use (H&E stain, 400X). This pigment affects oxidative stress, neurotransmitter imbalances, mitochondrial dysfunction, inflammatory responses, and liver damage (16). Studies have indicated a functional disorder in liver enzymes (ALP, AST, ALT). This viral defect is seen as the ability of the red pigment E129 to stimulate the micro-examination and peroxides to a great extent, and is active in eliminating free radicals and weakening antioxidant defense mechanisms, leading to oxidative stress and damage (17). While total protein levels, albumin and globulin decrease (18). This toxicity is attributed to the oxidizing effect of the red dye E129, leading to liver toxicity and the destruction of most liver cell membranes. Several studies have described lipid permeability in the myocardium and liver cells, caused by reactive oxygen species (ROS), as the primary cause of tissue damage induced by the dye E129. (8)

Regarding the histological analysis of the liver in group G3 in Figure 3, the group that received doses of *Annona muricata* (sourdough) leaf extract at a concentration of 400 mg/kg showed no signs of disease or apparent defects, and the central vein tissue, hepatocytes, and hepatic sinuses appeared normal. This is consistent with the results of group G1 in Figure 1. (19). The extracts reduced the toxicity of ALT, AST, and ALP in the livers of rats and also improved liver function. (20).

A section of the liver from G4 rats, treated with *Annona muricata* soursop leaf extract at a dose of 400 mg/kg body weight four hours before exposure to the red dye E129, showed no adverse effects, and the liver cells exhibited normal shape and structure (21). The protective properties of the antioxidants in *Annona muricata* leaf extract may be responsible for protecting the liver against damage caused by the red dye E129. Therefore, soursop leaves possess protective properties and safeguard the liver against damage caused by the red dye E129.

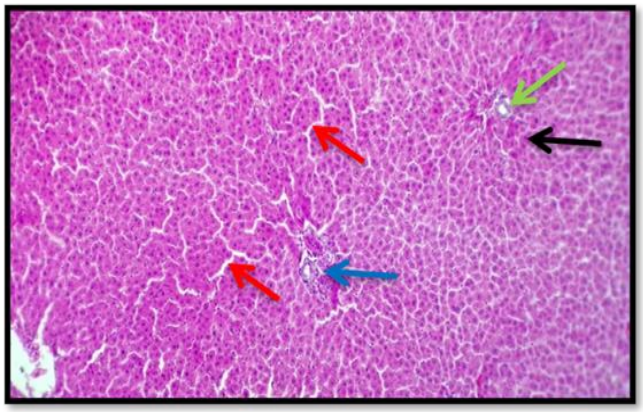

Figure 1. Microscopic histological section of the liver (control group). The hepatocytes are arranged in radial cords (black arrow). The hepatic sinuses are clearly visible (red arrow). There are several small dark areas, representing the pyloric regions (blue arrow) and the central vein (green arrow) (hematoxylin and eosin stain, 100x magnification).

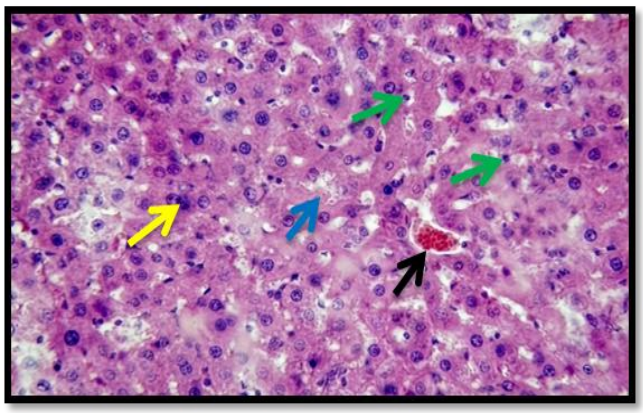

Figure 2. A microscopic image of liver tissue showing vacuole degeneration (yellow arrow), focal necrosis of hepatocytes (blue arrow), and nuclear shrinkage (green arrow). The section shows sinus dilation and congestion of microvascular blood vessels (black arrow). These degenerative changes indicate severe injury to hepatocytes. Hematoxylin and eosin staining, 400x magnification.

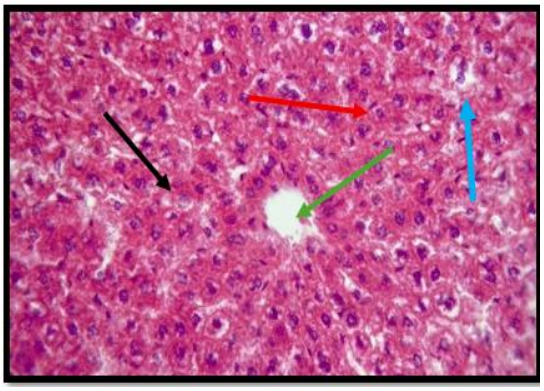

Figure 3. A microscopic histological section of the liver showing normal tissue with its normal structural integrity. Hepatocytes appear in radial cords (black arrow). Hepatic sinuses are clearly visible (red arrow). Several small dark areas are present, representing the pyloric region (blue arrow) and the central vein (green arrow) (hematoxylin and eosin staining, 400x magnification).

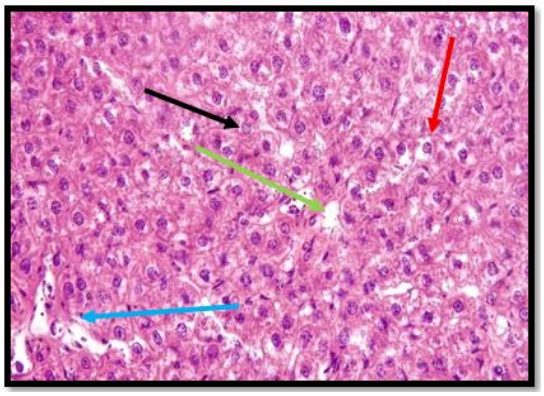

Figure 4. Microscopic histological section of the liver showing normal tissue. Hepatocytes appear in radial cords (black arrow). Hepatic sinuses are clearly visible (red arrow). Several small dark areas are present, representing the pyloric region (blue arrow) and the central vein (green arrow) (hematoxylin and eosin stain, 400x magnification).

Effect of the aqueous extract of soursop (*Annona muricata*) leaves on the activity of liver enzymes ALP, AST, and ALT in the serum of healthy male albino rats treated with Allura Red dye E129.

The results of the current study, as shown in Table (1), indicate a significant increase in the levels of liver enzymes (ALP, AST, ALT) in the group that was doused with red dye E129 at a concentration of 0.4 mg/kg every other day for 30 days, as follows: ALP (465.53±3.52), AST (339.35±3.37), ALT (139.09±1.51) compared with the negative control group G1 (262.69±3.69), (137.60±2.12), (50.76±0.56) respectively. Our current study did not record any significant differences ($P < 0.05$) in liver enzyme levels (ALP, AST, ALT) in group 3 (G3) (168.40 ± 3.04), (114.48 ± 1.00), and (44.60 ± 0.89), respectively, compared to the negative control group (G1). However, a significant decrease in liver enzyme levels (ALP, AST, ALT) was observed in group 3 (G3) compared to the positive control group (G2) (465.53 ± 3.52), (339.35 ± 3.37), and (139.09 ± 1.51), respectively.

The results of the current study also showed a significant decrease in the level of liver enzymes (ALP, AST, ALT) in the protective group G4, as follows: ALP (294.48±0.65), AST (151.78±0.85), ALT (55.00±0.72) compared to the positive control group G2, respectively.

Table 1. Effect of the aqueous extract of soursop fruit leaves on the activity of liver enzymes (ALT, AST, ALP) in the serum of healthy male albino rats treated with red dye E129

Treatment	Means ± stderr		
	ALT	AST	ALP
Negative control group G1 Water only	50.76 ± 0.56 C	137.60 ± 2.12 C	262.69 ± 3.69 C
G2 Control (positive): Red dye E129 0.4 mg/kg	139.09 ± 1.51 A	339.35 ± 3.37 A	465.53 ± 3.52 A
G3 Aqueous extract of soursop (*Annona muricata*) leaves Concentration 400 mg/kg	44.60 ± 0.89 D	114.48 ± 1.00 D	168.40 ± 3.04 D
G4 Aqueous extract of soursop leaves 400 mg/kg +Red dye E129 0.4 mg/kg	55.00 ± 0.72 B	151.78 ± 0.85 B	294.48 ± 0.65 B
L.S.D	2.9736	6.2977	8.9684

Liver enzymes (ALT, AST, ALP) are clinically important biomarkers for detecting liver toxicity, as their activity levels are primarily linked to overall health and the control of protein, carbohydrate, and fat metabolism (22).

Organs are sensitive to oxidants. The liver serves as a site for removing toxins and poisons from medications, thus raising their levels to normal when cellular damage is transferred to the bloodstream. In the kidneys, intestinal mucosa cells are used (23). Liver enzymes (ALT, AST, ALP) are clinically important biomarkers for assessing the extent of liver damage in cases of disease and poisoning. Their activity levels are primarily related to the body's health status and the regulation of protein, carbohydrate, and fat metabolism, and they reflect the integrity and function of liver cells (24). The results of the current study are consistent with those of study in which adult male rats were doused with the dye Laura Red E129 at a concentration of (50 mg/kg) every other day for 30 days (8).

They all pointed to a functional disorder in the liver represented by a significant increase in the levels of liver enzymes (ALP, AST, ALT) in the blood serum. This liver damage is attributed to the ability of the red pigment E129 to excessively stimulate the formation of free radicals and lipid peroxides, as well as inhibiting the efficiency of free radical removal systems and weakening antioxidant defense mechanisms, leading to oxidative stress and damage to liver cells. Elevated levels of liver enzymes result from the breakdown, damage, or necrosis of liver cells. These enzymes are released from within the liver cells into the bloodstream when cell membranes are damaged, leading to increased concentrations in the plasma. This may be attributed to increased plasma membrane permeability. Furthermore, the

release of these enzymes increases with the severity of the toxicity and the progression of various liver disorders and diseases (8).

The current results also showed that the significant increase in AST, ALP, and ALT activities and the pathological histological changes in the liver were due to the red dye E129. This toxicity is attributed to the oxidative effect of E129, leading to hepatotoxicity and the destruction of most liver cell membranes. Several studies have described lipid permeability in the myocardium and liver cells, caused by reactive oxygen species (ROS), as the main cause of tissue damage induced by E129 (25).

The current study also showed no significant differences ($P>0.05$) in liver enzyme levels (ALT, AST, ALP) in group (G3) compared to the negative control group (G1), and a significant decrease in group (G4) compared to the positive control group (G2). This is attributed to the presence of many biologically active compounds in soursop leaves, including acetogenin, which is characterized by its high efficacy against pathogens and its role in resisting diseases and types of cancer that occur in the body (26).

The results of the current study are consistent with those of Offor et al. (19), who demonstrated that oral administration of an aqueous extract of soursop leaves to male rats at doses of 200 mg/kg, 400 mg/kg, and 600 mg/kg for 30 days reduced liver enzyme levels. They are also consistent with the results of a study (27) .In their study of the effect of consuming an aqueous extract of soursop leaves at a concentration of (200, 400) mg/kg in male rats for 10 days, it showed significant protection against alcohol-induced liver tissue damage. A significant decrease in liver enzymes was observed, and it is believed that the aqueous extract of soursop leaves contributes to cell membrane repair and helps to bring liver enzyme concentrations (AST, ALT, ALP) within normal levels inside liver cells, either by increasing antioxidant levels or by increasing the level of antioxidants.

The results of the current study also agreed with Raj etal., (28) had concluded, as the results of their study indicated a significant decrease in the enzyme (ALP) due to the strong antioxidant activity of soursop fruit leaves, by working to raise antioxidant enzymes in the body by a large percentage in animals treated with the aqueous extract of soursop fruit and its role in reducing lipid peroxidation and controlling oxidative stress. as the study showed a decrease in liver enzymes due to its possession of many phytochemicals and biological activities that protect against liver injury (29).

CONCLUSION

These results indicate that the livers of rats exhibited a significant protective response when ingested with an aqueous extract of soursop (*Annona muricata*) leaves and fruit. This extract helped mitigate oxidative damage and histological changes caused by exposure to the red dye E129. This is attributed to its highly potent phenolic and flavonoid compounds, which are effective in combating oxidative stress.

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N/A

Conflict of Interest

The authors declare no conflict of interest.

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